

REMARKS

This reply is submitted in response to the Office Action dated November 15, 2002. Entry of the foregoing and reconsideration on the merits is respectfully requested. The attached appendix provides marked-up versions of the amended claims showing the changes that are made.

Amendment of the Claims:

Claims 1 and 27 are amended to recite a method that produces a population of cells comprising EG cells, support for which is found, for example, in the paragraph bridging pages 36-37. Claims 14, 26, and 30 are amended to recite, in step (iii), "transferring cells produced in step (ii) comprising EG cells into a recipient avian embryo," support for which is found in the specification, for example, on pages 39-40, which describe producing EG cells from the cultured PGCs. Claims 25, 28, and 29 are amended in step (iii) to specify transferring PGCs produced by step (ii) into a recipient embryo, for clarity. Claims 25 and 26 are amended in step (ii) to recite "said PGCs" instead of "such PGCs," and claim 26 is amended in step (iii) to recite transferring EG cells, also for clarity. Claims 25 and 26 are amended in step (iv) by deleting the phrase referring to the genotype of the PGCs, which is redundant. Claim 25 is further amended in step (ii) to recite that the PGCs are maintained in the recited tissue culture medium for a period of at least fourteen days, support for which is found in the specification, for example, at page 10, line 7. Claim 26 is further amended in step (iii) by deleting ", purified" from "isolated, purified PGCs," – the amendment filed June 3, 2002 showed this change in the initial statement of the amendment, but did not show it in the marked-up version of claim 26 in the Appendix. Claim 29 is also amended in step (iii) by deleting reference to "purified" PGCs. The last step of claims 26 and 30 is amended to recite a "recipient avian embryo containing said transferred EG cells," and the last step of claims 28

and 29 is similarly amended to recite a “recipient avian embryo containing said transferred PGCs,” for clarity. Claim 28 is further amended to recite in the preamble and step (iv) that the method produces a germline chimeric avian, support for which is found in the specification, for example, at page 18, lines 21-23. The above-described amendments of the claims do not add new matter.

Regarding Rejection of the Claims Under 35 U.S.C. §112, First Paragraph:

Claims 1-23 and 25-30 were rejected under 35 U.S.C. §112, first paragraph, on the grounds that the specification is enabling for:

- (1) a method for obtaining avian EG cells comprising:
 - (i) isolating primordial germ cells (PGCs) from an avian embryo;
 - (ii) culturing said PGCs for a period in tissue culture comprising LIF, bFGF, and IGF, such that avian EG cells are obtained,
 - (iii) transferring said EGs into a recipient avian embryo; and
 - (iv) obtaining a germline and somatic cell chimeric avian;

but the specification allegedly does not provide enablement for:

- (a) identifying avian EG cells in a mixed cell population of avian EG cells and PGCs;
- (b) stably transfecting avian EG cells, or
- (c) making chimeric avians that express exogenous proteins or have a non-wild-type phenotype.

The Applicants respectfully traverse these grounds for rejection, and submit that the specification enables persons skilled in the art to practice the claimed methods successfully without having to perform undue experimentation.

In regard to the requirement that the specification enable one to identifying avian EG cells in a mixed cell population of avian EG cells and PGCs; the Applicants respectfully submit that the invention as claimed can be practiced successfully without having to identify specific EG cells or PGCs in a population comprising EG cells. The specification teaches that the presence of EG cells in a population of cultured PGCs can be detected by transferring cells produced by culturing PGCs step into a recipient avian embryo, and obtaining somatic and germline chimeric avians. This is, in effect a functional assay for the presence of EG cells in a population of cultured PGCs. Identification of specific EG cells is not required by to practice the claimed method. Moreover, the specification also teaches that MC-480 antibody reacts strongly with EG cells, but not with PGCs (page 42). While an assay for detecting EG cells based on MC-480 has a subjective component, it is well within the ability of persons skilled in the art to determine the reactivity of known PGCs, and to use that value as a control to identify EG cells in a preparation of cultured PGCs that showing significantly greater reactivity with MC-480 than control PGCs. The Applicants respectfully submit that the Examiner's contention that a person skilled in the art would not be able to utilize the greater affinity of MC-480 for EG cells to distinguish a population of cultured PGCs comprising EG cells from a population of PGCs free of EG cells is incorrect.

The Applicants respectfully traverse the ground of rejection that the claims are not enabled because the specification does not disclose uses for somatic chimeric avians that are not germline chimeras. The specification does not have to disclose, and preferably omits, that which is well known by those in the art. In re Buchner, 929 F.2d 660, 661; 18 USPQ2d 1331, 1332 (Fed. Cir. 1991); Hybritech, Inc. v. Monoclonal Antibodies, Inc., 802 F.2d 1367, 1384; 231 USPQ 81, 94 (Fed. Cir. 1986); see also M.P.E.P. § 2164.01. Those skilled in the art recognize that each chimeric avian produced by injection of pluripotent EG cells into a recipient embryo is unique. The EG cells may give rise to both somatic and germ cells, so

that the chimeric avian is a germline chimera, or they may give rise to one or more somatic tissues, but fail to provide germ cells, so that the chimeric avian is a somatic, non-germline avian. The claimed method inherently produces both type of chimeric avians, and the claims properly encompass a method that results in both type of chimeras. The specification provides detailed description of making and using germline chimeric avians because there is great commercial interest in developing methods for producing these. While production of somatic chimeric avians that are not germline chimeras does not have the same commercial importance, one skilled in the art nonetheless would know that methods for making somatic chimeric avians are useful, for example, for studying avian development and the interactions of genetically different cell types within an individual avian. The interest of those in the art in such studies is shown by Watanabe et al. (Development, 1992, 114(2):3331-338), who described making somatic chimeric avians by inserting blastoderm cells into recipient avian embryos, and studying the chimeric avians to determine the relationship between the type of tissue formed by the injected cells, and the stage of the recipient embryo at the stage of injection. In addition to using somatic chimeras to study embryonic development and cell-cell interactions, those skilled in the art would know that somatic chimeric avians may be used for the same purposes as non-chimeric birds; e.g., as food. Accordingly, the Applicants respectfully request reconsideration and withdrawal of the rejection under 35 U.S.C. §112, first paragraph, on the ground that the specification does not teach uses for non-germline chimeric avians produced according to the claimed invention.

The Applicants respectfully traverse the assertion in the Office Action that the specification does not enable the claimed methods comprising stably transfecting avian cells and making chimeric avians that express exogenous proteins or have an altered phenotype. The Office Action states that the specification does not enable the use of transiently transfected avian EG cells, and that neither the specification nor the art available at the time

of filing teach how to use the transfected PGCs to produce transgenic avians that have an altered phenotype or that make exogenous proteins that can be isolated, which the Office Action asserts are the only uses for transfected PGCs disclosed in the specification.

In support of the rejection of the claims under 35 U.S.C. §112, first paragraph, as being non-enabled, the Office Action refers to the Applicants' disclosure in the specification that stably transfected PGCs have not been produced, and states that as neither the specification nor the art available at the time of filing describe making stably transfected EGs and using these to make transgenic avians, undue experimentation would have been required by one skilled in the art to determine how to use the transfected EGs of the claimed invention.

The Office Action further states that the only disclosed use for the claimed transfected EGs is to make transgenic birds that have an altered phenotype or produce heterologous protein that can be recovered. Wall (1996) is cited as describing a number of factors that make it impossible to predict the effect that expression of an exogenous gene in a transgenic avian will have on the phenotype of the transgenic bird. For example, the position in the genome at which the transgene inserts, the interaction of host proteins that regulate gene expression with regulatory nucleic acid sequences in the inserted DNA, and the flanking host chromosomal DNA, all contribute to the unpredictability of both the level of expression, and the tissue-specific pattern of the expression of a transgene in a transgenic avian. The Office Action states that because of the unpredictability of the expression of a transgene in a transgenic avian, one skilled in the art would have had to perform undue experimentation in order to use transfected EGs.

The Applicants submit that the specification describes the claimed invention in such terms and in sufficient detail that at the time the application was filed, a skilled individual would have been able to follow the teachings of the specification to make transfected EGs according to the claimed method without undue experimentation. As discussed below

Neither the recognized unpredictability of transgene expression in a transgenic avian, nor the recognized difficulty of stably transfecting avian cells with plasmid DNA expression constructs, would have made it necessary for one of skill in the art to perform undue experimentation in order to make or use the claimed invention. Furthermore, as discussed below, one skilled in the art at the time the invention was filed would also have known how to use the claimed transfected EGs to make transgenic, chimeric avian embryos and transgenic avians without undue experimentation. Therefore, the rejection of the claims as being non-enabled is improper and should be withdrawn.

The rejected claims are drawn to a method of making transfected avian EGs derived from PGCs that are cultured in vitro for a prolonged period (i.e., at least 14 days) in the absence of feeder cells according to the disclosed invention. Key to the claimed invention is the disclosed method for culturing avian PGCs in vitro for a prolonged period in the absence of feeder cells. Until the present invention, it was believed that long-term culture of avian PGCs in vitro required growing the PGCs on a feeder cell layer (for example, see Pain et al., 1996, page 2345).

Prior to the present invention, those skilled in the art were familiar with methods for transfecting PGCs, and inserting the transfected PGCs into avian embryos to produce transgenic, chimeric avian embryos and transgenic avians. For example, Vick et al. (Proc. R. Soc. Lond., 1993, 251:179-182, copy provided previously) described using a retroviral vector to stably transfect avian PGCs, and injecting the transfected PGCs into recipient embryos to produce transgenic birds, as discussed in the Applicants' reply to the first Office Action. Although Vick et al. did not report that they detected expression of the transgene in transgenic avians, they taught that studying the transgenic, chimeric birds produced by their methods can provide information about their reproductive biology, because the retroviral DNA inserts in an apparently random fashion, so that it is possible to distinguish individual

transfected PGCs from each other in the gonad of a transgenic avian by the site of insertion of the foreign DNA (see page 182). Thus, one skilled in the art would have recognized that transgenic, chimeric avians such as those disclosed by Vick et al. are useful even in the absence of transgene expression.

Moreover, given the advanced state of the art of genetic engineering at the time the application was filed, one of skill in the art would reasonably have regarded the report by Vick et al. of using retrovirally transfected avian PGCs to produce a transgenic avian with 4.5 kilobases of stably integrated foreign DNA as strong evidence undue experimentation would not have been required to practice the claimed method by making and using transfected EGs to produce transgenic embryos and avians in which a transgene is successfully expressed. The Vick et al. reference was the first report of the use of transfected PGCs to produce a transgenic avian. As stated by the authors on page 181, the aim of their work was "to demonstrate the feasibility of using primordial germ cells as a means of gaining access into the avian genome." Prior to publication of the Vick et al. reference, Bosselman et al. (J. Reprod. Fert., 1990, 15E:200; see also, U.S. Patent No. 5,162,215) and Lee et al. (Proc. 4th World Cong. Genet. Livestock Prod., 1990, 16:107-110) had already demonstrated successful expression and inheritance of retrovirally introduced transgenes in transgenic avians, as described on pages 118-119 of Simkiss (1994, pages 106-137). In addition, Chen et al. (J. Reprod. Fertil. Suppl. 41:173-182) reported in 1990 that a retrovirally introduced transgene encoding bovine growth hormone was successfully expressed in transgenic chickens that grew larger and matured more rapidly than wild type birds. Accordingly, those skilled in the art understood at the time the application was filed that a transgene introduced by a retroviral vector into cells of a transgenic avian could be stably integrated into the avian genome and expressed at sufficient levels as to affect the phenotype of the host avian.

In describing the work of Vick et al., Simkiss states (pages 121-122):

“... It is clear, therefore, that PGCs can be used as vehicles for introducing foreign DNA into the genome of the bird, and subsequent experiments have been directed at characterizing this phenomenon and increasing its efficiency.

The protocol for producing transgenics by such a procedure would, in outline, consist of (a) isolating PGCs, (b) integrating foreign DNA into their genome, (c) screening the cells for suitable gene expression, (d) introducing these genetically manipulated cells into a recipient embryo to form a chimera and (e) breeding from this chimera and selecting those offspring that contained the introduced genes.”

Following publication of Vick et al., Allioli et al. (1994) reported successful expression of the lacZ reporter gene in isolated avian PGCs cultured and retrovirally-transfected in vitro, as noted in Applicant’s previous response. By the time the present application was filed, retroviral vectors were used routinely for obtaining expression of transgenes in avian primary cell cultures and in embryonic chick tissues in ovo (see Bell et al., Mol. Biotechnol., 1997, 7(3)289-298). The characterization of the results reported by Vick et al. in the Simkiss (1994) review article described above, and reports of stable integration and successful expression of retrovirally introduced transgenes in transgenic avian cells in vitro and in vivo provide clear evidence that one skilled in the art at the time the application was filed would have regarded the Vick et al. references as showing that transgenic PGCs could be used to produce stably transfected transgenic avians in which transgene expression was reasonably expected to occur.

The Office Action states that the specification fails to enable one skilled in the art to use the claimed invention because the levels and patterns of expression of transgenes introduced into transgenic avians by the claimed invention are unpredictable. The Applicants

respectfully traverse this ground of rejection, because it imposes limitations that are not recited in the claims. The specification states that objects of the invention are “to introduce desired nucleic acid sequences into avian embryonic germ cells which have been obtained by culture of PGCs for prolonged periods in tissue culture,” and to use transfected EG cells “for the production of transgenic, chimeric avians” (page 7, lines 2-13). The specification is directed at those skilled in the art, who recognized the significance of the inventor’s discovery and knew how to use it. The rejected claims recite a method for producing transfected avian EGs that encompasses making EGs in which there is very little or even no detectable expression of the transgene. As taught by Vick et al. and as discussed above, one skilled in the art would have known that stably transfected avian EGs could be used to study avian reproductive biology, e.g., by using the inserted DNA to identify individual transgenic cells and their lineages in a chimeric avian, even when expression of the transgene is undetectable. Moreover, as discussed further below, at the time the application was filed, those skilled in the art recognized that it was not necessary to be able to predict the level or pattern of transgene expression, or the change in phenotype caused by expression of a transgene in a transgenic avian, in order to find utility in the transgenic avian or in the method and cells by which it is generated.

The Office Action asserts that the claimed methods are not enabled, because introduction of plasmid DNA expression constructs into avian PGCs or EGs is expected to result in transient transfection. At the time the application was filed, the difficulties associated with producing transgenic avians that are discussed in the Office Action were generally recognized by those skilled in the art. Simkiss (1994) taught that “during the development of procedures using PGCs it is most convenient to use vectors that are likely to give the highest rates of integration and at the present time these are retroviruses,” and that as of 1992, stable transfection of avian cells had only been achieved with retroviral vectors (pp.

129-130). In 1994, Love et al. reported making stably transfected transgenic chickens by microinjecting plasmid DNA into avian embryos (Biotechnology, 12(1):60-63, abstract attached). However, as discussed below, most attempts to introduce non-retroviral DNA into avian cells appear to have produced transiently transfected cells, and at the time the application was filed, methods comprising transiently transfecting avian cells, including PGCs, and inserting the transfected cells into recipient embryos to generate transgenic avians in which the transgenes are expressed, were widely used by those skilled in the art. They recognized that transgenic avians in which transgenes are expressed, even if only transiently, are highly useful for studying the tissue-specific and developmental stage-specific properties of promoters and other nucleic acid sequences that regulate gene expression, and for studying the relationship between the level or pattern of transgene expression in tissues of a transgenic avian embryo or chick and any changes in phenotype caused by transgene expression. For example:

- Wagner et al. (Clin. and Exper. Hypertension, 1995, 17(4):593-605) described using transgenic animals to analyze promoter regulatory elements for tissue-specific expression, and to identify cis-acting factors that control gene transcription (p. 594).
- Simkiss (1994) taught that the avian embryo is "probably the best-studied example of vertebrate development" (p. 107), and described a study by Perry et al. (1991) of the temporal and spatial expression of the lacZ gene in avian embryonic cells following microinjection of cloned reporter constructs into the germinal disc of a freshly fertilized avian egg (see pages 114-115).
- Wall (1996) taught that, in view of the unpredictability of transgene expression, the best way to test the operability of a DNA expression construct in a tissue of a transgenic animal is to introduce the construct into an animal of the species of interest and assay its expression in the target tissue in vivo (p. 62).

- Naito et al. (VI International Symposium on Avian Endocrinology, March 31-April 5, 1996) reported transfecting plasmid DNA containing a lacZ gene into cultured avian PGCs in vitro, and inserting the transfected PGCs into recipient embryos to produce transgenic, chimeric avian embryos with gonads in which there were PGCs in which the lacZ gene was expressed, and they stated that their method could be used to test the expression of exogenous DNA in the gonads of developing chick embryos.
- Demenix et al. (Biotechniques, 1994, 16(3):496-501) described transfecting plasmid DNA expression constructs encoding reporter genes under control of various promoters into chick embryos in vivo and measuring the tissue-specificity and temporal aspects of the level of transgene expression in cells of the developing embryo.
- Kelder et al. (Gene, 1989, 76(1):75-80) transfected a plasmid DNA expression construct encoding a reporter gene under control of an inducible promoter into cultured avian cells and measured the dependence of transgene expression on the concentration of inducer in the cell culture medium.
- Rosenblum et al. (Transgenic res., 1995, 4(3):192-198) used cationic liposomes to transfect plasmid DNA expression constructs encoding reporter genes under control of a RSV promoter into avian embryonic cells, they measured changes in the level of transgene expression in the transfected embryos over time, and reported that while stable integration of transfected DNA appeared to be a rare event, liposome-mediated transfection of embryos is useful for studying promoter activity in vivo and may be useful for transfecting genes to study embryonic development.
- Ono et al. (Exp. Anim., 1995, 44(4):275-278) injected cationic liposomes complexed with plasmid DNA expression constructs into avian embryos and transfected the PGCs in vivo, as evidenced by detection of transgene expression in the gonads at later embryonic stages.

o In similar experiments, Watanabe et al. (Mol. Reprod. Dev., 1994, 38(3):268-274) injected avian embryos with cationic liposomes complexed with plasmid DNA expression constructs encoding a lacZ reporter gene under control of either a RSV promoter or a chicken beta-actin promoter, obtained transfection of avian PGCs and other cell types in vivo, and then measured and compared the levels of transgene expression directed by the two different promoters in the transfected tissues of the developing embryos, including in transfected PGCs that migrated to the gonads of the developing embryos.

The Federal Circuit has long held that 35 U.S.C. §112 does not require a specific disclosure of that which is already known to one of ordinary skill in the art. Case v. CPC International, Inc., 221 USPQ 196, 201 (Fed. Cir. 1984). The claimed invention corresponds closely to the stated objects of the invention, “to introduce desired nucleic acid sequences into avian embryonic germ cells which have been obtained by culture of PGCs for prolonged periods in tissue culture,” and to use such transfected EG cells “for the production of transgenic, chimeric avians.” The published scientific articles described above show that at the time the application was filed, one skilled in the art would have known how to use the claimed methods for producing avian EGs that are transfected either transiently or stably with a DNA expression vector, and how to use the transfected EGs to make transgenic, chimeric avian embryos and transgenic avians. For example, one skilled in the art would know that transgenic, chimeric avian embryos and transgenic avians produced by the claimed invention are useful for studying tissue-specific promoter activity and the effects of transgene expression in the developing avian embryo, as discussed above.

The present invention facilitates methods for transfecting avians that were long-recognized as being needed by those skilled in the art. Simkiss (1994) discussed the feasibility of transfecting isolated avian PGCs in vitro and using the transfected PGCs to make transgenic avians, and the desirability of methods that would facilitate this approach;

for example, to permit the screening of transfected PGCs in vitro for suitable gene expression (see pages 121-122). Simkiss stated that at the time of writing (1992), PGCs could only be cultured for short periods, and that a method permitting long-term culture of PGCs in vitro was needed to facilitate genetic manipulation of PGCs for production of transgenic, germ line chimeras (p. 124). Vick and Simkiss stated on page 181, column 2, that “clearly it is the number of primordial germ cells that can be obtained rather than their cellular maturity that determine[d] the success rate in forming these [transgenic] embryos.” Vick and Simkiss obtained a higher rate of success using PGCs from the germinal crest before their vascular migration because they were able to isolate more PGCs. Simkiss (1994) also discusses the possibility that a method for long-term culturing of PGCs might give rise to embryonic germ cells that could be used to produce chimeric germ-line avians (page 123). As Applicants discussed in their replies to the earlier Office Actions, one skilled in the art would reasonably have expected that the disclosed methods for long-term culture of avian PGCs would make it easier to practice the methods for transfecting and using PGCs described in the prior art. Using the present invention, Vick et al. could have obtained as many cells as they needed, and because the present invention allows for long term culturing of cells, it would have permitted screening the transfected PGCs prior to chimera production, as suggested by Simkiss. Moreover, since the present method generates EG cells, one skilled in the art would have recognized that methods such as those used by Vick and Simkiss, in combination with the disclosed methods for producing pluripotent EG cells, could be used to produce transgenic chimeric somatic and germ-line avians, as speculated by Simkiss.

Those skilled in the art at the time the application was filed recognized that transiently transfected PGCs and EGs could be used to make transgenic, chimeric embryos and chicks that can be studied to determine tissue-specific and developmental stage-specific activities of nucleotide sequences that regulate gene expression, and to determine the effects of transgene

expression on avian development, as discussed above. The level of skill in the art is high. Scientific articles such as Simkiss et al. expressly described a need for the development of methods such as the claimed method that permits long-term culture of PGCs in vitro in order to facilitate the genetic manipulation of PGCs and EGs for production of transgenic, somatic and germ line chimeras, also discussed above. Accordingly, the Applicants submit that at the time the application was filed, one skilled in the art would have been able to follow the teachings of the specification and practice the claimed methods for producing transfected avian EGs and for using these to produce transgenic, chimeric avian embryos and transgenic avians, without having to perform undue experimentation. The Applicants therefore respectfully request reconsideration and withdrawal of the rejections under 35 U.S.C. §112, first paragraph.

Regarding Rejection of the Claims Under 35 U.S.C. §112, Second Paragraph:

Claims 1-23 and 25-30 were rejected under 35 U.S.C. §112, second paragraph, as being indefinite, because the metes and bounds of avian primordial germ cells (PGCs), and of avian embryonic germ (EG) cells in a population containing PGCs cultured for a period of time in the presence of LIF, bFGF, SCF, and IGF, are not clear. The Applicants respectfully traverse this ground of rejection. Claims 1 and 27 are amended to recite production of a population of cells comprising avian EG cells; and in claims 14, 25, 26, and 28-30, step (iii) is amended to recite transferring cells produced by step (ii) into a recipient avian embryo. The claims recite methods for producing EG cells and for using PGCs and EG cells to produce chimeric avians. The specification teaches methods for using cultured PGCs to produce germline chimeric avians, and for using a cultured cell population comprising avian EG cells to produce somatic and germline chimeric avians. Neither the invention as described in the specification nor the claims as written require active identification of specific

EG cells or PGCs, and one skilled in the art would clearly understand the metes and bounds of the claims as written. Withdrawal of this ground for rejection under 35 U.S.C. §112, second paragraph, is therefore respectfully requested.

Claims 25 and 26 were rejected under 35 U.S.C. §112, second paragraph, as being indefinite, because the meaning of “such PGCs” in step (ii) of claims 25 and 26, and of “said PGCs” in step (iii) of claim 25, is allegedly unclear, and because the method of claim 25 does not produce germline chimeric avians as recited in the preamble. The Applicants respectfully submit that these grounds for rejection under 35 U.S.C. §112, second paragraph, have been removed by the foregoing amendment. In particular, claims 25 and 26 are amended in step (ii) by replacing “such PGCs” with “said PGCs,” which clearly refers to the isolated PGCs recited in step (i). Step (iii) of claim 25 is amended to specify transferring PGCs produced by step (ii) into a recipient avian embryo; and claims 25 and 26 are amended in step (iv) by deleting the phrase referring to the genotype of the PGCs, so that the PGCs obtained by the claimed method are described in the same terms as in the preamble.

Claim 29 was rejected under 35 U.S.C. §112, second paragraph, as being indefinite, because there is no antecedent basis for “purified” PGCs recited in step (iii). The Applicants respectfully submit that this ground for rejection of claim 29 under 35 U.S.C. §112, second paragraph, has been removed by the amendment of claim 29 that deleted the reference to “purified” PGCs in step (iii).

Claim 3 was rejected under 35 U.S.C. §112, second paragraph, as being indefinite, because there allegedly is no antecedent basis for the phrase “minimum amounts” in claim 2. The Applicants respectfully traverse this ground of rejection, which was overcome by the amendment of claim 3 in the reply filed June 3, 2002.

Claims 25 and 26 were rejected under 35 U.S.C. §112, second paragraph, as being indefinite, because the preambles of claims 25 and 26 allegedly recite an improved method,

the nature of which is not described in the body of the claims. The Applicants respectfully traverse this ground of rejection, which was overcome by the amendment of claims 25 and 26 in the reply filed June 3, 2002.

Claim 25 was rejected under 35 U.S.C. §112, second paragraph, as being indefinite in reciting “desired phenotype,” which lacks antecedent basis and the meaning of which is unclear. The Applicants respectfully traverse this ground of rejection, which was overcome by the amendment of claim 25 in the reply filed June 3, 2002.

Claims 26 and 30 were rejected under 35 U.S.C. §112, second paragraph, as being indefinite in reciting “said cultured population of primordial germ cells” and “said isolated, purified PGCs,” which lack antecedent basis. The Applicants respectfully traverse this ground of rejection, which was overcome by the amendment of claims 26 and 30 in the reply filed June 3, 2002. The term “isolated PGCs” in step (iii) clearly refers to the PGCs isolated in step (i).

Claims 26 and 30 were also rejected under 35 U.S.C. §112, second paragraph, as being indefinite because “said recipient embryo” recited in step (iv) allegedly lacks antecedent basis. The Applicants traverse this ground of rejection, as the recipient embryo recited in step (iv) is clearly that into which EG cells were transferred in step (iii). However, in order to expedite prosecution, claims 28 and 30 are amended to recite “said recipient embryo containing said transferred EG cells.” Withdrawal of the rejection of claims 26 and 30 under 35 U.S.C. §112, second paragraph, on this ground is respectfully requested.

Claims 26, 28, and 30 were rejected under 35 U.S.C. §112, second paragraph, as being indefinite for allegedly reciting the phrase “express a phenotype.” The Applicants respectfully traverse this ground of rejection, which was overcome by the amendment of the claims in the reply filed June 3, 2002.

Claim 27 was rejected under 35 U.S.C. §112, second paragraph, as being indefinite for allegedly reciting the phrase “the EG cells,” the meaning of which is unclear. The Applicants respectfully traverse this ground of rejection, which was overcome by the amendment of claim 27 in the reply filed June 3, 2002.

Claim 28 was rejected under 35 U.S.C. §112, second paragraph, as being indefinite for allegedly reciting the phrase “said purified PGCs,” which lacks antecedent basis. The Applicants respectfully traverse this ground of rejection, which was overcome by the amendment of claim 28 in the reply filed June 3, 2002.

In view of the foregoing, reconsideration and withdrawal of the rejections under §112, second paragraph is respectfully requested.

Regarding Rejection of Product Claims 21 and 22 Under 35 U.S.C. §102:

Claims 21 and 22, rejected under 35 U.S.C. §102(b) and 102(e), were amended in the reply filed June 3, 2002, so that they are no longer drawn to an avian EG cell line. Accordingly, reconsideration and withdrawal of the rejections of claims 21 and 22 under 35 USC 102 is respectfully requested. The Applicants reserve the right to pursue claims to the products of the claimed invention in a different application.

Regarding Rejection of the Claims Under 35 U.S.C. §102 and 103 in view of Pain et al., alone or with Simkiss:

Claims 1, 3-11, 14-16, and 20-22 were rejected under 35 U.S.C. §102(b) on the grounds that the claimed method for prolonged culture of avian PGCs resulting in production of EG cells is anticipated under 35 USC 102(b) by Pain et al. (1996), as supported by Simkiss (1994). The examiner asserts that the claimed method for culturing PGCs for prolonged

periods is not distinct from the method for culturing avian stage X blastoderm cells in the absence of feeder cells described by Pain et al.

The Applicants submit that Pain et al. do not anticipate the claimed methods because they do not disclose or suggest the claimed method comprising culturing avian PGCs *in vitro* in medium containing LIF, bFGF, SCF, and IGF-1, for at least 14 days in the absence of feeder cells, nor do they disclose or suggest that prolonged culture of avian PGCs *in vitro* under such conditions will result in production of pluripotent EG cells. In fact, Pain et al. and other prior art references expressly teach away from long-term culture of avian PGCs in the absence of feeder cells.

The Pain et al. reference described evaluating the effects of various growth factors on cultured avian embryonic cells by culturing chicken and quail cells from dissociated stage X blastoderms *in vitro* in medium containing LIF, bFGF, SCF, and IGF-1 in the presence and absence of feeder cells for 3-5 days, and then scoring or counting the alkaline phosphatase-positive colonies (page 2341, lower right column; and Fig. 2 on page 2342). Pain et al. reported that under their conditions, the feeder cells “strongly promoted the development of alkaline phosphatase-positive colonies (page 2341, bottom of right column). Pain et al. also disclosed studying the importance of LIF in long-term culture of chicken embryonic cells (CECs) by culturing the CECs for 11 passages in medium (“ESA complete medium”) containing LIF, bFGF, SCF, and IGF-1, and then culturing the cells for an additional 24 days in the same medium with or without LIF (page 2343, paragraph bridging columns, and Fig. 4). Pain et al. reported that LIF is required for the long-term growth of avian embryonic cells and the expression of antigens characteristic of ES cells, and that avian embryonic cells expressing antigens characteristic of ES cells could be maintained for at least 35 passages, i.e. more than 160 days, in the presence of LIF. The paragraph on page 2343 of Pain et al. describing the long-term culturing of CECs does not specify whether the long-term cultures

of CECs in medium containing LIF, bFGF, SCF, and IGF-1 were carried out using feeder cells; however, Pain et al. clearly stated on page 2345 (right column) that their long-term culture conditions included the use of feeder cells:

“The culture conditions which included the use of mouse embryonic feeder cells and the inclusion of LIF, IL-11, SCF, bFGF, IGF-1, and ARMA in the medium, facilitated the proliferation of cells with an undifferentiated phenotype during more than 35 passages, i.e., more than 160 days.”

In summary, Pain et al. described culturing PGCs *in vitro* in medium containing LIF, bFGF, SCF, and IGF-1 in the presence and absence of feeder cells, they reported that the cells grew much better in the presence of feeder cells, and they taught that PGCs could be cultured for a prolonged period in medium containing LIF, bFGF, SCF, and IGF-1 in the presence of feeder cells. Nothing in Pain et al. or Simkiss suggests the claimed invention comprising culturing avian PGCs *in vitro* in medium containing LIF, bFGF, SCF, and IGF-1, for at least 14 days in the absence of feeder cells, and nor does either reference, alone or in combination, suggest that prolonged culture of avian PGCs *in vitro* under the claimed conditions will result in production of pluripotent avian EG cells. Accordingly, reconsideration and withdrawal of the rejection under 35 USC 102(b) is respectfully requested.

Claims 1 and 2 were rejected under 35 U.S.C. §103(a) on the grounds that the claimed method for prolonged culture of avian PGCs resulting in production of EG cells is anticipated under 35 USC 102(b) by Pain et al. (1996), as supported by Simkiss (1994), as set out in the previous Office Action. The previous Office Action stated that Pain et al. disclosed obtaining EG cells after long-term culturing of PGCs, as evidenced by production of germline and somatic cell chimeras after introduction of the into stage X chicken embryos. The Office Action asserted that the description by Pain et al. of short-term culture conditions used to test

the effects of different combinations of growth factors would have motivated one of ordinary skill in the art to use the conditions of the claimed method in order “to optimize the conditions required to obtain EG/ES cells.”

The Applicants respectfully traverse the rejection of claims 1 and 2 under 35 U.S.C. §103(a) as set forth in the present and previous Office Actions. In the first place, the previous Office Action mischaracterizes the method described by Pain et al. as the prolonged culture of avian PGCs. Pain et al. cultured blastoderm cells from a Stage X embryo which, in view of Simkiss, would have been expected to include a small percentage of cells that were PGCs, and they reported obtaining a mixed, non-clonal culture of cells capable of contributing to ectoderm and, with low frequency, also capable of populating the germ line (page 2346, right column). In providing explanations for the low frequency of germline transmission that they observed with the cultured cells obtained by their method, Pain et al. noted that they were unable to maintain clonal growth of chicken embryo cells (CECs), and that germline precursor cells may actually have been preferentially lost under their culture conditions (page 2346, right column).

As discussed above, the only disclosure in the Pain et al. reference of culturing avian embryonic cells in the presence and absence of feeder cells was the description of a short-term in vitro method for evaluating the effects of various growth factors on cultured avian embryonic cells, in which cells from dissociated stage X blastoderms were cultured in vitro in medium containing LIF, bFGF, SCF, and IGF-1 in the presence and absence of feeder cells for 3-5 days, and then scored for production of alkaline phosphatase-positive colonies (page 2341, lower right column; and Fig. 2 on page 2342). In describing the results they obtained from this method, Pain et al. actually taught away from the claimed invention, in reporting that feeder cells “strongly promoted the development of alkaline phosphatase-positive colonies (page 2341, bottom of right column), and that their long-term culture conditions

included the use of feeder cells (see page 2345, right column). One of ordinary skill in the art would also have known that other research groups had found feeder cells to be necessary in long-term culturing of avian embryonic cells in vitro; for example, see Petite et al., U.S. Patent No. 5,340,740.

Thus, the cited prior art references did not provide any suggestion or motivation to one of ordinary skill in the art to practice the claimed method by culturing avian PGCs in vitro in medium containing LIF, bFGF, SCF, and IGF-1, for at least 14 days in the absence of feeder cells; nor did the prior art suggest culturing avian PGCs in vitro under the claimed conditions for a prolonged period to obtain pluripotent avian EG cells, with any expectation of success. Accordingly, the claimed methods would not have been obvious to one of ordinary skill in the art in view of Pain et al., taken with Simkiss, at the time the application was filed, and the Applicants respectfully request that the rejection under 35 U.S.C. §103(a) be reconsidered and withdrawn.

Regarding Rejection of the Claims for Double Patenting:

Claims 1-8, 14-16, and 20-22 of the present application were rejected for obviousness-type double patenting over claims of U.S. Patent No. 6,159,569, alone, or in view of Pain et al. Claims 1-8, 21, and 22 of the present application were also rejected for obviousness-type double patenting over claims of co-pending U.S. Application No. 09/127,624, alone or in view of Pain et al.

A Terminal disclaimer is submitted herewith that disclaims the term of a patent that issued for the present claims with respect to the terms of U.S. Patent No. 6,159,569, and co-pending U.S. Application No. 09/127,624. Accordingly, the Applicants respectfully request that the rejection for obviousness-type double patenting be withdrawn.

The issues raised by the Final November 15, 2002, have been addressed in this and the foregoing Amendments and Replies. It is respectfully submitted that the present application is in a condition for allowance and a Notice to that effect is earnestly solicited. If the Examiner has any further questions or issues to raise regarding the subject application, it is respectfully requested that he contact the undersigned so that such issues may be addressed expeditiously.

Respectfully submitted,
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APPENDIX

IN THE CLAIMS:

Claims 1, 14, and 25-30 are amended as shown below:

1. (Four Times Amended) A method of obtaining avian embryonic germ (EG) cells comprising:

- (i) isolating primordial germ cells (PGCs) from an avian embryo; and
- (ii) culturing said PGCs in the absence of a feeder layer in a culture medium comprising
 - (1) leukemia inhibitory factor (LIF),
 - (2) basic fibroblast growth factor (bFGF),
 - (3) stem cell factor (SCF) and
 - (4) insulin-like growth factor (IGF),

so that a population of cells comprising avian EG cells [are] is obtained.

14. (Four Times Amended) A method of producing chimeric avians comprising:

- (i) isolating primordial germ cells (PGCs) from an avian;
- (ii) culturing the PGCs in the absence of a feeder layer in a tissue culture medium containing at least the following growth factors;
 - (1) leukemia inhibitory factor (LIF),
 - (2) basic fibroblast growth factor (bFGF),
 - (3) stem cell factor (SCF) and
 - (4) insulin-like growth factor (IGF)

for a sufficient time to produce embryonic germ (EG) cells;

(iii) transferring cells produced by step (ii) comprising said EG cells into a recipient avian embryo; and

(iv) obtaining a germline and somatic cell chimeric avian.

25. (Thrice Amended) A method of producing germline chimeric avians comprising:

(i) isolating primordial germ cells (PGCs) from a Stage XII-XIV avian embryo;

(ii) maintaining [such] said PGCs for a period of at least fourteen days in a tissue culture medium containing at least the following growth factors:

- (1) leukemia inhibitory factor (LIF),
- (2) basic fibroblast growth factor (bFGF),
- (3) stem cell factor (SCF) and
- (4) insulin-like growth factor (IGF);

(iii) transferring [said] PGCs produced by step (ii) into a recipient avian embryo;

and

(iv) obtaining germline chimeric avians [having germline cells that have the genotype of said PGCs].

26. (Four times Amended) A method of producing germline and somatic cell chimeric avians which comprises:

(i) isolating primordial germ cells (PGCs) from a Stage XII-XIV avian embryo;

(ii) maintaining [such] said PGCs in a tissue culture medium containing at least the following growth factors:

- (1) leukemia inhibitory factor (LIF),
- (2) basic fibroblast growth factor (bFGF),

- (3) stem cell factor (SCF) and
 - (4) insulin-like growth factor (IGF),
- for a sufficient time to produce embryonic germ (EG) cells;
- (iii) transferring cells produced by step (ii) comprising said EG cells into a recipient avian embryo of the same species as the avian used to obtain said isolated[, purified] PGCs;
 - (iv) allowing said recipient avian embryo containing said transferred EG cells to develop into a germline and somatic cell chimeric avian [having germline and somatic cells that have the genotype of said PGCs].

27. (Thrice Amended) A method for producing avian embryonic germ (EG) cells comprising:

- (i) isolating primordial germ cells (PGCs) from a Stage XII-XIV avian embryo;
- (ii) culturing said PGCs for a period of at least fourteen days in tissue culture in the absence of a feeder layer in a culture medium comprising:
 - (1) leukemia inhibitory factor (LIF),
 - (2) basic fibroblast growth factor (bFGF),
 - (3) stem cell factor (SCF) and
 - (4) insulin-like growth factor (IGF)

so that a population of cells comprising avian EG cells [are] is produced.

28. (Thrice Amended) A method for producing a germline chimeric avian comprising:

- (i) isolating primordial germ cells (PGCs) from a Stage XII-XIV avian embryo;

- (ii) culturing said PGCs for a period of at least fourteen days in tissue culture in the absence of a feeder layer in a culture medium comprising:
 - (1) leukemia inhibitory factor (LIF),
 - (2) basic fibroblast growth factor (bFGF),
 - (3) stem cell factor (SCF) and
 - (4) insulin-like growth factor (IGF);
- (iii) transferring said PGCs produced by step (ii) into a recipient avian embryo of the same species as the avian used to obtain said isolated PGCs;
- (iv) allowing said recipient avian embryo containing said transferred PGCs to develop into a germline chimeric avian.

29. (Thrice Amended) A method for producing a germline chimeric avian comprising:

- (i) isolating primordial germ cells (PGCs) from a Stage XII-XIV avian embryo;
- (ii) culturing said PGCs for a period of at least fourteen days in tissue culture in the absence of a feeder layer in a culture medium comprising:
 - (1) leukemia inhibitory factor (LIF),
 - (2) basic fibroblast growth factor (bFGF),
 - (3) stem cell factor (SCF) and
 - (4) insulin-like growth factor (IGF);
- (iii) transferring said PGCs produced by step (ii) into a recipient avian embryo of the same species as the avian used to obtain said isolated [, purified] PGCs;
and
- (iv) allowing said recipient avian embryo containing said transferred PGCs to develop into a germline chimeric avian.

30. (Thrice Amended) A method for producing germline or somatic cell chimeric avians comprising:

- (i) isolating primordial germ cells (PGCs) from a Stage XII-XIV avian embryo;
- (ii) culturing said PGCs for a period of at least fourteen days in tissue culture in the absence of a feeder layer in a culture medium comprising:
 - (1) leukemia inhibitory factor (LIF),
 - (2) basic fibroblast growth factor (bFGF),
 - (3) stem cell factor (SCF) and
 - (4) insulin-like growth factor (IGF)₁for a sufficient time to produce embryonic germ (EG) cells;
- (iii) transferring said cells produced by step (ii) comprising EG cells into a recipient avian embryo of the same species as the avian used to obtain said isolated PGCs; and
- (iv) allowing said recipient avian embryo containing said transferred EG cells to develop into a germline or somatic cell chimeric avian.